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Induced breeding and larval rearing of *Monopterus cuchia* (Hamilton, 1822) under the agro-climatic conditions of Meghalaya, India

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Abstract
Monopterus cuchia is a fresh water eel widely distributed in Nepal, Myanmar, Pakistan but predominantly available in Bangladesh and North East Region of India. The population of the species has been declined in its natural habitat to a great extend due to various environmental and anthropogenic factors. So far no report was available regarding artificial propagation of the species *M. cuchia,* especially from India. Hence it is necessary to develop an induced breeding protocol by standardized doses of synthetic hormone for artificial propagation of the fish.Thus, an experiment was set up for induced breeding of the species using synthetic hormone, Gonopro-FH. After hormonal administration (Gonopro-FH), brooders were released into specially prepared ponds and spawning was natural and spontaneous. During the period of analysis, fecundity of *M. cuchia* was calculated as 924 eggs (length= 78cm, weight= 344 gms). Induced breeding was successful using Gonopro-FH and the final dosage was 1ml/kg body weight to the female along with 0.3ml/kg body weight to the male. Final fertilization percentage was 75-80% and hatching percentage was 80-85%.

Keywords: Fecundity, Gonopro-FH, Induced breeding, Meghalaya, *Monopterus cuchia*

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INTRODUCTION

Monopterus cuchia (Hamilton, 1822) is one of the most nutritional, medicinal and tasty fish with high market demand. This species is locally known as *Khabsein* in Khasi language (Meghalaya). *M. cuchia* is an economically important fresh water fish, widely distributed in India, Nepal, Bangladesh, Pakistan and Myanmar (Menon, 1999; Mirza and Alam, 2002; Zhou *et al*., 2002). International Union for Conservation of Nature (IUCN) Red List of Bangladesh has enlisted *M. cuchia* in their red list of vulnerable fishes of Bangladesh (IUCN, 2015). Mud Eel is available only in selected pockets of Meghalaya, India viz. Khweng and Nongpoh (Ri-Bhoi District), Shella (East-Khasi Hills District) and Baghmara (Garo Hills District). It has developed specialized pharyngeal pouches for bimodal gas exchange (Hughes and Munshi, 1973; Munshi, 1985; Begum *et al*., 2017) and thus they can survive in mud and oxygen poor water of its habitat. It was observed that the population of the species has declined day by day from different areas of Bangladesh due to indiscriminate harvest (Begum *et al*., 2017) and similar pattern also observed in North East India including Meghalaya.

Hence, need of the hour is for replenishment of stock by artificial propagation for conservation and culture. The fish can play a unique role for socio-economic welfare of the area and thus social fishery could be developed (Quddus *et al*., 2000).

Mud Eel can be identified by the analysis of some important morphometric characters. Species exhibits sexual dimorphism only during breeding season. It was observed that mature female was larger than male fish and the abdomen of female fish is swollen and brownish in colour with rough abdominal skin. Anus and genital pore was observed as tubular in male and round shape in female (Miah *et al*., 2013). In case of females, *M. cuchia* contain a single lobe of ovary. The ovary lobe is connected along their dorsal surfaces by a thin mesentery from which they are suspended in the abdominal cavity. Lobe is elongated and found to be cylindrical size and the anterior part of the ovary is more or less cylindrical. In males, the testis is soft and elongated structures lying in the body cavity and ventral to the swim bladder. It leads posterio-ventrally into vas deferens that unites to form a spermatic duct opening to the

exterior through the urogenital aperture. Testis attached to the dorsal body wall by the connective tissue, mesorchium and composed of numerous thin walled seminiferous lobules (Chakraborty, 2018). *M. cuchia* is nocturnal in nature and the fish is considered to be carnivorous by feeding habit. They feed on earthworms, frogs, tad-poles, small aquatic animals, shrimp, crayfish and also on detritus (Narejo *et al*., 2003).

Moreover, the feeding items found in the gut content of *M. cuchia* mainly includes the Annelids (such as the Earthworms, Sludge worms, Leeches etc.), Copepods, Cladocerans (viz. *Bosmina, Sididae* etc), Rotifera, Insect larvae, Insect body parts, Sand, Mud as well as some unidentified species. Due to the presence of noticeable amount of sand and mud in gut content of this species, this suggested that *M. cuchia* is a bottom feeder (Kurbah and Bhuyan, 2018). Density and diversity of *M. cuchia* are decreasing day by day due to indiscriminate harvest from nature by using various pesticides and by drying up of water bodies.

Consequently, broods of *M. cuchia* are decreasing alarmingly and breeding grounds became squeezed (Begum *et al*., 2017). Induced breeding is a technique whereby ripe fish breeders are stimulated by pituitary hormone or any other synthetic hormone introduction to breed in captive condition. The stimulation promotes timely release of sperms and eggs (Paul and Chanda, 2014). Artificial propagation is the primary means of getting fish seed for culture in confined water as well as conservation in its natural habitat. Though, attempt has been made for induced breeding of *M. cuchia* in Bangladesh (Begum *et al*., 2017), but till now no attempt has been made for artificial propagation of the fish from India. Therefore, it is necessary to develop artificial breeding and nursing techniques of *M. cuchia*. Hence the present work was aimed to formulate the protocol for artificial propagation of *M. cuchia* under agro-climatic conditions of Meghalaya, India using a synthetic hormone.

MATERIALS AND METHODS

The induced breeding of *M. cuchia* was conducted during April-September 2016. During this period, several attempts were made by trial and error methods with different doses of hormone to check the effect of the hormone in better result using synthetic gonadotropin hormone, trade named Gonopro-FH. The experiments were conducted at the hatchery in the Department of Fishery Science, St. Anthony's College and repeated attempts were made for another two years i.e. 2017 and 2018. Collection of brooders was done during the month of February 2016 from three different areas of Meghalaya viz. Khweng and Nongpoh (Ri -Bhoi District), Shella (East Khasi Hills District) and Baghmara (Garo Hills District). The brooders were carried to Shillong in a Bolero Pickup vehicle and the distance travelled varied from 34.3 km to 255.3 km. Brooders were treated with a potassium permanganate ($1mgl^{-1}$) for 3 minutes and then released into the specially managed cemented cistern of the hatchery complex. The cisterns were prepared by adding approximately 10cm of mud at the bottom collected from another pond of the hatchery complex. The second layer was prepared by adding chopped straw about 10cm in thickness. Third layer composed of finely chopped banana trunk with 10cm in thickness. Fourth layer consists of cow dung and the fifth and top most layers was prepared by adding mud in a slope with one end higher than the other. After that, water was introduced into the cistern 15cm above the top layer and allows all the materials to decompose for one week. Water in the cisterns was replenished every fifteen days to remove froth and any other unwanted materials present in the pond. The brooders were fed with rice mixed with jaggery at the ratio of 1:0.05 in gram in addition to earthworms, tadpoles, and coarsely powdered thrash fishes. Moreover, water quality parameters viz. Dissolved Oxygen, Free Carbon Dioxide, Total Hardness and Total Alkalinity of pond water was analyzed and assessed by using American Public Health Association Standard methods for examination of water and waste water (APHA, 2005).

Brooders were selected for injection and weight of the specimens was recorded. Both males and females brooders were segregated one day before injection. A total of 68 brooders (i.e. 32 males and 36 females) were selected for breeding. Gonopro-FH was administered near the head portion of the specimens. Artificial breeding of *M. cuchia* was performed in the evening time of a sunny day since this species is nocturnal. In order to determine the final optimum dose for successful breeding of the fish, a series of experiments was performed by trial and error methods. Additionally, fertilization, fecundity and hatching percentages were analyzed and calculated by simple random sampling and counting methods. Fecundity of the species was calculated and determined by Bagenal (1978).

RESULTS

Physico-chemical parameters: In order to obtain a healthy fish population of *Monopterus cuchia*, maintenance of water quality was pre-requisites. From the experiment conducted, it was observed that mean Physico-Chemical Parameters of the water was Dissolved Oxygen (7.42 mg/L), pH (7.2), Free Carbon dioxide (0.1 mg/L), Total Alkalinity (66.75 mg/L) and Total Hardness (83.16 mg/L). Analysis of water characteristics round the year was important especially in cemented ponds

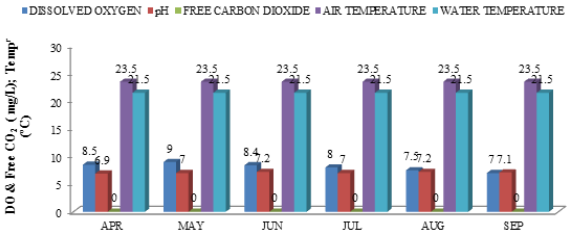

Fig. 1. *Showing average Dissolved Oxygen, pH, Free Carbon dioxide, air-temperature and water temperature of cultured ponds during breeding period.*

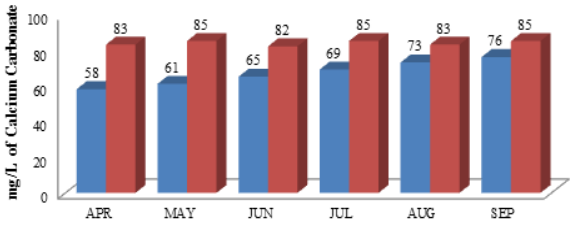

Fig. 2. *Showing average Total Hardness and Total Alkalinity of cultured ponds during breeding period.*

Table 1. Showing detail result of trial and error experiments with administration of Gonopro-FH hormone to *M. cuchia.*

Hormone used	Number of trials	Single dose (ml/kg body weight)		Results
		Female 36	Male 32	
Gonopro-FH	I	0.5	0.2	No response
	II	0.6	0.2	No response
	III	0.7	0.25	Matured eggs but not fertilized
	IV	0.8	0.25	Fertilization percentage (10-20%)/ No hatching takes place
	V	0.9	0.3	Fertilization percentage (30-40%)/ Hatching percentage (10-20%)
	VI	1.0	0.3	Fertilization percentage (75-80%)/ Hatching percentage (80-85%)
	VII	2.0	0.3	Spawning affected/ blocked vent in female

Table 2. Showing the final doses of Gonopro-FH for protocol of induced breeding of *M. cuchia* in Meghalaya.

Hormone	Single dose (ml/kg body weight)		
	Temperature range: 22ºC-25ºC		
	Female	Male	Results
Gonopro-FH	1.0	0.3	Fertilization percentage: 75-80% Hatching percentage: 80-85%

for maintenance of healthy brooder. The water quality parameters of cultured ponds during breed period are shown in Figs. 1 and 2.

Induced breeding: Fecundity was calculated before induced breeding experiments and observed out that the fecundity of *M. cuchia* was 924 eggs (length= 78cm, weight= 344 gms). Administration of Gonopro-FH to the brooders was given on a sunny day during evening time (April-September) to avoid sudden fall of temperature which may affect spawning (Chaudhuri, 1963; Alikunhi *et al*., 1964). After several trial and errors throughout the experiment periods, final dose of hormone for induced breeding of the species was determined. The final protocol with specific doses of synthetic hormone Gonopro-FH for artificial propagation of *M. cuchia* has been represented in Table-1. The first trial was done with a dosage of 0.5 ml/kg body weight to female brooders along with 0.2ml/kg body weight to the male but no response was observed in this case. Further, second trial was done with an increase in hormonal injection of 0.6ml/kg body weight only to the female whereas in case of male same dosage was given i.e. 0.2ml/kg body weight; no response was observed during this period of analysis. Third trial was done with an increasing hormonal administration of 0.7ml/kg body weight to the female along with an increasing dosage of 0.25ml/kg body weight in case of male. It was observed that the eggs were found to be matured but they are not fertilized. For the fourth trial, the hormonal dosage was again increased up to 0.8ml/kg body weight of Gonopro-FH to the female brooders whereas same amount was administered in case of male i.e. 0.25ml/kg body weight. During this period of analysis, it was found out that the eggs were observed to be fertilized but they are not hatched. Moreover, the fertilization percentage was found out to be very less i.e. 10-20% fertilization. Furthermore, a fifth trial was conducted followed by the administration of 0.9ml/kg body weight to the female along with 0.3ml/kg body weight to the male. In this case it was observed that the eggs were found to be fertilized but partially hatched. Moreover, the fertilization percentage was less i.e. 30-40% but the hatchlings were not survived. From the experiment conducted, it was also observed the hatching percentage was 10-20% i.e. the percentage of hatching was very less and the rate of survivability was also very low. Low survivability rate may be due to sudden drop in temperature since the ponds used are cemented tanks and therefore pond temperature drop down because of cold. Since it is difficult to check the spawns inside the mud, therefore survivability rate may be low. After this, sixth trial was conducted with an increased in Gonopro-FH dosage up to 1ml/kg body weight to the female along with 0.3ml/kg body weight to the male and observed that fertilization and hatching percentage is better. Fertilization percentage in this case was 75-80% and the hatching percentage was ob-

served to be 80-85% and hence survivability rate was more. Seventh trial was done with a hormonal administration of 2.0ml/kg body weight to the female and 0.3ml/kg body weight to the male. However, it was found that hormonal dosage of 2.0 ml/kg body weight blocked the female vent and thereby affecting fish spawning. This indicates that further increased in Gonopro-FH does not have any effect on fertilization process and moreover fertilization was not successful.

The final doses of synthetic hormone for the induced breeding protocol developed for the fish *M. cuchia* was finalized based on the trial and error methods using Gonopro-FH and represented in Table-2.

DISCUSSION

A study on physico-chemical parameters is essential in order to know the component of water present in the pond for optimum growth of aquatic organisms and life in aquatic environment largely governed by physico-chemical parameters and their stability. This fish adapt themselves to survive in changed environment for their own existence. Determinant of good fish growth and their well being in water body includes dissolved oxygen, pH, free carbon dioxide, total hardness, total alkalinity, and temperature etc. As time passes by, the concentrations of physico-chemical parameters changes due to human activities and lack of environmental regulations.

In order to determine the growth and well being of the species, it is necessary to monitor the temperature. Hence, proper knowledge and maintenance of water temperature is essential for fish culture. Administration of Gonopro-FH to the brooders was given on a sunny day during evening time to avoid sudden fall of temperature. Maintenance of temperature is essential for successful induced breeding of *M. cuchia*. High temperature affects the hatching of eggs and also might lead to mortality of spawns. But if the temperature is less than 21ºC, eggs survivability will be less. Temperature also affects the metabolism of fish. So higher and lower optimum temperature affect the food intake of the fish. Therefore temperature has a role to play in stunted growth and sexual maturity of the fish. Thus this experiment was conducted when air temperature ranged 22-25ºC and water temperature ranged 21-22ºC.

Another important physico-chemical parameter for analyzing water quality is dissolved oxygen. Dissolved oxygen (DO) is related to phytoplankton production and respiration (Smith and Piedrahita, 1988), nitrogen waste such as ammonia is related to the amount of organic matter inputs and ammonium excretion by fish (Gross *et al*., 1999; Hargreaves, 1998) and water temperature and thermal stratification are controlled by sunlight and air temperature (Egna and Boyd, 1997; Losordo and Piedrahita, 1991). Adequate Dissolved Oxygen concentrations in a fish pond are essential for maintaining optimal growth (Chang and Ouyang, 1988). From the experiment conducted, the minimum and maximum value of Dissolved Oxygen was found to be 6.2- 9.0 mg/L with an average value of 7.42mg/L. Similar findings was also reported by (Sandhya and Benarjee, 2016) on the study of physico-chemical properties of some selected fresh water fish ponds in relation to fish production in Warangal area. Dissolved Oxygen is a measure of the amount of gaseous oxygen dissolved in an aqueous solution that plays a vital role in biology of cultured organisms. The concentration of dissolved oxygen in this case was found to be within the optimum range and maintained in fish ponds at all time.

To analyze whether water is acidic, neutral or basic can be determined by pH and continuous variation in pH can cause stress, poor growth and increase mortality rate of the species. pH indicates the acid-base balance of cultured pond water. Survival and growth of fish also depends on the pH of the water. Minimum pH value in this case was observed to be 6.5 and maximum value was found to be 8.5. From the experiment conducted it was observed that the mean pH value was found out to be 7.2 indicates that the pH in the cultured ponds lies within the optimum range. Similar finding was also reported by (Tucker and D'Abramo, 2008) on the study of managing high pH in fresh water ponds where pH equal to 7.0 and this describes the neutral point of water at which the concentrations of hydrogen and hydroxyl ions (OH-) are equal (each at 10-7 moles/L). If the pH value is less than 7 (pH<7), it indicates that the pond water is not suitable for fishes. If the pH is more than 7 (pH>7), fish might die or may lead to stunted growth. Carbon dioxide can be removed by chemical treatment of pond water with liming agents such as quicklime, hydrated lime or sodium carbonate. These liming agents chemically react directly with carbon dioxide, resulting in reduced Carbon dioxide and increased Alkalinity and pH. The daily pattern of Carbon dioxide concentration is generally opposite that of Dissolved Oxygen. During the day, algae take up or "fix" Carbon dioxide that is free in the water and Carbon dioxide concentration is therefore lowest (often 0 mg/L) during late afternoon, when dissolved oxygen is highest (Hargreaves and Brunson, 1996). This report is similar with the present findings since the amount of Free Carbon dioxide present in the cultured ponds of *Monopterus cuchia* was very less with an average value of 0.1mg/L. Free Carbon dioxide in water is the by-product of metabolism. More than a particulate level, carbon dioxide present in the water is toxic to the life in water. Water quality is a group of physical, chemical and biological factors which

influence the use of water for the purpose of fish culture (Sandhya and Benarjee, 2016). One of the important factors of water quality is the Total Alkalinity. Alkalinity is the sum of negative ions reacting to neutralize hydrogen ions when an acid is added to water. During this period of analysis, it was observed that minimum Total Alkalinity was found to be 58 mg/L and maximum value was observed to be 77 mg/L with an average value of 66.75 mg/L and this concentration of alkalinity in ponds water was taken care by proper liming. According to (Bhatnagar and Devi, 2013) optimum Alkalinity for fish productivity is between 25 to 100 mg/L. From the experiment conducted, the values obtained were within this ranged which makes these ponds suitable for fish farming. Ideal value of Total Alkalinity for fish culture should not be less than 50mg/L. Water hardness is a measure of the alkaline earth metals such as Calcium and Magnesium concentration in water samples (Ehiagbonare and Ogundiran, 2010). Hardness depends on the dissolved solids and pH of the water. The minimum amount of Total Hardness present in cultured pond was found to be 80 mg/L and the maximum amount was observed to be 85 mg/L with an average value of 83.16mg/L. This revealed that the amount of Total Hardness lies within the optimum range and similar findings was also reported by (Wurts and Durborow, 1992) on the study of interactions of pH, Carbon dioxide, Alkalinity and Hardness in fish ponds and reported that Hardness ranged between 25-100 mg/L is good for fish culture. Hardness gives a measure of the total concentration of the divalent metallic cations like Calcium, Magnesium and Strontium. Proper liming can rectify the hardness. Ideal value for fish culture is 30-180mg/L.

The habitat of this species is fresh and salty water as they are mainly found in shallow well vegetated water and mud. They often spend their daytime hiding under stones and mud as they have a burrowing habit (Nasar, 1997). During dry season approximately, 4-6 eels were found to take shelter inside a single hole. But during breeding season, one male and one female share one hole.

Moreover, this species breed once a year i.e. April -September. The reason was due to delay maturation of females when reared them under captivity in comparison with the maturation of males. The availability of lower number of matured females also contributes to the problem. Induced breeding of Mud Eel (*M. cuchia*) was successful using a synthetic hormone i.e. Gonopro-FH. The final dosage for successful induced breeding of *M. cuchia* with Gonopro-FH hormone was found to be 1ml/kg body weight to the female along with 0.3ml/kg body weight to the male. After injection of both male and female, the brooders were released into the ponds and spawning was natural and spontaneous. After analyzing and assessing the breeding behaviour of this species, it was observed that the eggs were found to be fertilized and hatched and both the fertilization percentage as well as the hatching percentage was high. Fertilization percentage was observed to be 75-80% and the hatching percentage was found to be 80-85% and hence survivability rate was more. This species do not release eggs outside their nest holes and while investigating the fertilized eggs inside the pit, eggs are released in relatively wider portion of their holes. The results observed was similar to reports of some induced breeding of other fishes using synthetic hormones (Bhuyan *et al*., 2002; De Silva *et al*., 2004; Achionye- Nzeh and Obaroh, 2012; Angel *et al*., 2015; Motilan *et al*., 2014; Ghosh *et al*., 2012). The analysis proved that synthetic hormone is more effective in induced breeding of the fish. However, further study should be continued for the determination of micro changes in hormonal dosage for large scale production of fish seeds. The advantages of synthetic hormones is that they are cost effective, less time consuming, easily available in the market, ready to use in liquid form, they are more stable with long shelf life, consistent potency and assured in breeding response. Gonopro-FH is an agent used to promote or facilitate fish reproduction. Before conducting any induced breeding protocol, feeding is one of the most important functions of an organism in order to be healthy and productive. Basic functions such as growth, development, reproduction etc. all take place at the expense of the energy, which enters the organism in the form of its food. Feeding activity influences the growth and productivity of fishes. Feeding is the dominant activity of the entire life cycle of fish (Joadder and Hossain, 2008). During this period, the brooders were fed with rice bran, oil cake, flour, earthworms, snails, tadpoles, dry fish powder, rice, vitamins, minerals and small aquatic insects etc. Ideal temperature for proper feeding and growth of *M. cuchia* is between 20-35ºC and the fish will not feed if the average temperature is at or below 12ºC. Moreover, feed intake by this species increased with the increasing in temperature and decreased with the decreasing in temperature. The collected spawns were also fed with live feed, dry fish powder, boiled broken rice and egg yolk mixed with boiled rice.

Conclusion

Practically no work was available regarding artificial breeding of *M. cuchia* especially from Meghalaya, thus, this present investigation provides new information on successful artificial propagation using synthetic hormones. Correct weight of the brooders is important to administer right amount of hormone, other wise, either there will be no responses in spawning or brooders might die. If the brooders are stressed, then

Gonopro-FH may not have positive effect on the fish. The positive response of *M. cuchia* to the synthetic hormone and formulation of a protocol for artificial propagation will go in a long way for quality seed production of the fish in the country. Mass production of seeds and conservation of the species through use of hormone and manipulating the environment with proper management is required to save this fish from extinction.

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